Microbiota within the perennial ice cover of Lake Vida, Antarctica

Annika C. Mosier, Alison E. Murray & Christian H. Fritsen

Desert Research Institute, Division of Earth and Ecosystem Sciences, Reno, NV, USA

Correspondence: Christian H. Fritsen, Desert Research Institute, Division of Earth and Ecosystem Sciences, 2215 Raggio Parkway, Reno, NV 89512, USA. Tel.: +1 775 6737487; e-mail: chris.fritsen@dri.edu

Present address: Annika C. Mosier, Department of Geological and Environmental Sciences, Stanford University, Stanford, CA, USA.

Received 1 May 2006; revised 23 August 2006; accepted 29 August 2006.
First published online 9 November 2006.

DOI:10.1111/j.1574-6941.2006.00220.x

Editor: Max Häggblom

Keywords
perennially ice covered lake; Antarctica; McMurdo Dry Valleys; microbial assemblages; phylogenetic diversity.

Abstract

Lake Vida, located in the McMurdo Dry Valleys, Antarctica, is an 'ice-sealed' lake with ~19 m of ice covering a highly saline water column (~245 ppt). The lower portions of the ice cover and the lake beneath have been isolated from the atmosphere and land for *circa* 2800 years. Analysis of microbial assemblages within the perennial ice cover of the lake revealed a diverse array of bacteria and eukarya. Bacterial and eukaryal denaturing gradient gel electrophoresis phylotype profile similarities were low (< 59%) between all of the depths compared (five depths spanning 11 m of the ice cover), with the greatest differences occurring between surface and deep ice. The majority of bacterial 16S rRNA gene sequences in the surface ice were related to *Actinobacteria* (42%) while *Gammaproteobacteria* (52%) dominated the deep ice community. Comparisons of assemblage composition suggest differences in ice habitability and organismal origin in the upper and lower portions of ice cover. Specifically, the upper ice cover microbiota likely reflect the modern day transport and colonization of biota from the terrestrial landscape, whereas assemblages in the deeper ice are more likely to be persistent remnant biota that originated from the ancient liquid water column of the lake that froze.

Introduction

Many of the earth's perennially ice covered lakes are located in the McMurdo Dry Valleys, Antarctica (e.g. Lakes Bonney, Fryxell, Joyce, Miers, Vanda and Vida). These lakes are ice-covered (3–6 m on average) year round because of continuously cold air temperatures (annual average temperature $-20\,^{\circ}C$, Wharton *et al.*, 1993). The thickness of ice is typically controlled by sublimation off the top ($\sim 30\,cm\,year^{-1}$) and freezing on the bottom.

Several of the perennially ice covered lakes within the McMurdo Dry Valleys sustain biotic life. Microbiota have been described within the perennial ice covers of these lakes (Fritsen & Priscu, 1998; Priscu *et al.*, 1998; Doran *et al.*, 2003) and their water columns (Ward & Priscu, 1997; Voytek *et al.*, 1999; Stackebrandt & Brambilla, 2002; Glatz *et al.*, 2006). During warmer summers when air temperatures $> 0\,^{\circ}C$ persist for several days, peripheral moats form around the edges of many of the ice covers (Wharton *et al.*, 1993). The ice-free margins, often several meters wide, allow glacial melt water (formed during periodic melting of glacial ice) and associated biota to recharge the lakes. Aeolian transport deposits organisms directly onto the surface of the ice covers.

Lake Vida is an atypical perennially ice-covered lake in the McMurdo Dry Valleys with *c.* 19 m of ice covering a highly saline liquid brine (Doran *et al.*, 2003). Organic matter locked within the Lake Vida ice at 12 m beneath the surface was radiocarbon dated at *circa* 2800 years old (Doran *et al.*, 2003). Incoming glacial melt water during warm summer months periodically floods the frozen surface. This flooding was observed in the 1980s (C.P. McKay, pers. commun.) and again during the austral summer of 2001/2002 (J.C. Priscu, pers. commun.). The thickness of the Lake Vida ice cover presumably prevents the glacial melt water from flowing beneath the ice cover. Consequently, the periodic flooding and freezing of the Lake Vida ice cover increases the thickness of the ice from the top surface, unlike other perennially ice-covered lakes in the Dry Valleys. A net freezing of $\sim 7\,cm\,year^{-1}$ also may occur on the bottom of the Vida ice cover (Doran *et al.*, 2003). To date, microbial community composition within the Lake Vida ice cover or brine pocket had not been evaluated.

Biotic distribution and diversity were examined in the ice cover of Lake Vida to augment the paucity of data on microbial communities within perennial ice covers and to better understand the habitability of these ecosystems. Here, enumeration, diversity, and putative activity of microbial populations at five depths in the ice cover of Lake Vida are described. These results enable better resolution of the origin of microbiota in the Lake Vida ice cover and determination of whether the ice cover contains organisms indicative of the biota that may lie within the briny water column of the lake.

Materials and methods

Study site

Lake Vida is located in Victoria Valley (77°23′S, 161°56′E) in the McMurdo Dry Valleys, Antarctica (Fig. 1). Annual precipitation in the McMurdo Dry Valleys ranges from 5 to 10 cm year^{-1}, annual air temperature averages $-20\,^{\circ}$C, and humidity is $< 50\%$ (Matsumoto, 1993). Light levels fluctuate dramatically between the seasons, from continuous light in the summer to continuous darkness in the winter. The soils and many of the water bodies in the McMurdo Dry Valleys are saline due to concentration of marine salts derived from relict seawater in the region (Takamatsu *et al.*, 1998), as well as evaporitic concentration of salts (Matsumoto, 1993).

Lake Vida is 3.5 km long and 1 km wide with a surface elevation of 390 m above sea level. The lake is covered year-round by ~19 m of ice. Ice temperatures increase from *c.* $-25\,^{\circ}$C at the surface of the ice cover to $-10\,^{\circ}$C near the bottom (at 15.9 m, Doran *et al.*, 2003). The brine pocket beneath the ice (detected by ground-penetrating radar) remains liquid at *circa* $-10\,^{\circ}$C, which infers a salinity of > 245 ppt (Doran *et al.*, 2003). Chloride concentrations increase dramatically near the bottom of the ice (from 0.4 to 62.2 mg L^{-1} above 12.5 m to a striking 235.9 mg L^{-1} at 15.9 m), suggesting that this ice was originally derived from the brine layer (Doran *et al.*, 2003). During warm summers, melt water from Victoria Upper and Lower Glaciers and smaller glaciers in the St Johns Range floods the frozen lake (Calkin & Bull, 1967). Doran *et al.* (2003) described five sediment layers, two sand layers and three microbial mat layers distributed within the ice cover above ~12 m. Lake Vida has no surface outflows.

Fig. 1. Location of Lake Vida, Antarctica. Landsat image of ice-covered Lake Vida located within the McMurdo Dry Valleys, courtesy of and adapted from NASA's Visible Earth (http://visibleearth.nasa.gov/).

Sample collection and processing

Two ice cores (15.9 and 14 m) were collected from Lake Vida in 1996 using a Polar-Ice Coring Office 4-inch electromechanical ice-coring rig. Cores were kept frozen at $-20\,^{\circ}$C until processing in 2003. Core segments containing enough volume of melt water for subsequent analyses were chosen to represent a range of depths within the portion of the ice cover that is frozen year-round (below ~2 m). Five sections of the 15.9-m ice core were used for this study: 4.67–4.82, 6.7–6.86, 11.6–11.8, 14.47–14.65, and 15.5–15.85 m beneath the surface of the ice cover. Herein, these core segments are referred to by the bottom depth of each section: 4.8, 6.9, 11.8, 14.7, and 15.9 m.

Because of our intent to recover biota and DNA from the ice, core sections were handled aseptically in a UV and ethanol-sterilized laminar flow hood in a 4 °C cold room. Core sections were cleaned by removing an average of 21.75% of the ice core mass by rinsing with sterile 3% hydrogen peroxide (H_2O_2). This was followed by a thorough rinse with sterile water to remove remnant H_2O_2. In an experiment designed to test the effectiveness of this cleaning method, pseudo-ice cores were intentionally contaminated with a bacterial isolate from a Lake Fryxell microbial mat and then cleaned with 3% H_2O_2. Most (99.2%) of the total contaminant cells (determined by microscopy) were removed from the cleaned pseudo-ice cores and 100% of the culturable cells (determined by viable cell counts) were removed. In a comparable experiment conducted with sterile 97% ethanol, cleaning removed 99.5% of the contaminant cells determined by total cell counts and only 75% those determined by viable cell counts (i.e. culturable cells).

Clean core sections were melted at 4 °C in sterile Whirl-Pak® bags or HDPE containers. Ice-melt water was immediately subsampled for direct counts, chlorophyll *a* measurements, and biomass for nucleic acid extraction. Subsamples for direct cell counts were preserved with sterile-filtered glutaraldehyde (1% final concentration) and stored at 4 °C until filtration within an average of 18 days (maximum of 47

days). Melt water (50–250 mL) was filtered onto 25 mm Whatman 0.4-μm pore size GF/F filters for chlorophyll *a* analysis and frozen at −80 °C. Melt water (60–780 mL) for nucleic acid extraction was syringe-filtered through 0.2-μm pore size Supor (Gelman Scientific) sterivex filters. Filter cartridges were filled with 1.8 mL of sucrose lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose) and frozen at −80 °C.

Chlorophyll *a* analysis, enumeration and sizing

Chlorophyll *a* from biomass collected on GF/F filters was extracted with cold 90% acetone overnight. Fluorescence (F*o*) was determined with a Turner Designs 10AU Fluorometer calibrated with pure chlorophyll *a* (Sigma Corp.) (Welschmeyer, 1994). Direct bacterial counts were determined by epifluorescent microscopy (Hobbie *et al.*, 1977) using the generalized nucleic acid DAPI (4′,6-diamidino-2-phenylindole) stain. Cell sizes (20 cells per depth) were determined with IMAGE-PRO PLUS (Media Cybernetics Inc.) image analysis software calibrated with fluorescent beads (Duke Scientific Corp.). Cell biovolume (μm^3) was based on mean length and width of spheres (cocci) or cylinders with hemispherical caps (rods) (Simon & Azam, 1989).

Environmental genomic DNA extraction and PCR amplification

DNA extraction methods (Pospiech & Neumann, 1995; Massana *et al.*, 1997; Sheridan *et al.*, 2003) were tested for robustness by extracting nucleic acids from gram-positive cell cultures (*Rhodococcus* sp.). Additionally, the Massana *et al.* (1997) and Pospiech & Neumann (1995) methods were compared on a duplicate Vida ice section. The enzymatic and lysis method (Massana *et al.*, 1997) had comparable nucleic acid recovery values, amplified better using PCR (brighter PCR bands), and produced more bacterial denaturing gradient gel electrophoresis (DGGE) bands on the duplicate ice sample. DNA from the Lake Vida ice sections was extracted using this method as previously described (Massana *et al.*, 1997). Nucleic acids were quantified using the PicoGreen (Molecular Probes Inc.) dsDNA quantitation method.

PCR amplifications were carried out in an automated thermal cycler (Applied Biosystems). PCR mixtures contained 0.1–10 ng of template DNA, 1 × PCR reaction buffer, 0.2 mM dNTPs, 1 μM of each primer, 2.5–3.5 mM Mg^{2+} and 0.5 U Taq DNA polymerase. Initially, samples were screened with primers targeting the three domains of life using primers Bact27F (Lane, 1991) and Univ1492R (Kane *et al.*, 1993) for bacteria, Arch20F (Massana *et al.*, 1997) and Arch958R (DeLong, 1992) for archaea, and Euk1A (Diez *et al.*, 2001) and Univ1492R for eukaryotes. Mixed template DNA was amplified for DGGE with bacterial (GC358F and 517R, Murray *et al.*, 1996) and eukaryal (960FbGC and 1200R, Lin *et al.*, 1996; Gast *et al.*, 2004) primers with hot start conditions (Erlich *et al.*, 1991). The thermal cycling program was similar to that described to Muyzer *et al.* (1993). In brief, the denaturing temperature began at 94 °C and decreased 2 °C every second cycle until annealing at 55 °C for 20 cycles, followed by primer extension at 72 °C for 7 min. Multiple PCR reactions were pooled (three reactions for eukarya and 8–11 reactions for bacteria) and purified with Microcon YM-100 concentrators (Millipore) for DGGE analysis. Bacterial clone libraries were generated by amplifying the 16S rRNA gene with 27F and 1492R primers. The conditions consisted of denaturation at 94 °C for 5 min, 30 cycles of 94° for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by extension at 72 °C for 7 min.

Eukaryal and bacterial DGGE analysis

Eukaryal and bacterial DGGE analyses were performed using the BioRad D Code™ System and methods detailed by Murray *et al.* (1996). Equal amounts of PCR product (800 ng for bacteria and 300 ng for eukarya) were loaded onto 8% (w/v) polyacrylamide gels, 1 mm thick, and run in 1 × TAE (20 mM Tris acetate, 10 mM acetate, 0.5 mM disodium EDTA). The denaturing gradient contained 25–55% denaturant for the eukaryal DGGE and 30–60% denaturant for the bacterial DGGE (100% denaturant corresponds to 7 M urea and 40% v/v, formamide). Electrophoresis proceeded for 1000 V-h. Gels were stained with 1 × SYBR Gold (Molecular Probes) for 10 min, viewed on a UV-transilluminator, and photo-documented with a Kodak EDAS 290 System.

Gel images were analyzed using GELCOMPAR II software (Applied Maths) in which bands were automatically detected and matched by the program. Band calling and matching was confirmed manually. In this study, DGGE was used to evaluate community changes between ice depths by analyzing shifts in banding patterns between samples. Pairwise comparisons of DGGE band similarities between samples were determined using Sorenson's index (Murray *et al.*, 1996): $C_s = 2j/(a+b)$, where j is the number of bands common to two samples, and a and b are the number of bands in samples A and B, respectively.

Numerous eukaryal DGGE bands were selected for sequencing. Bands were excised from gels with a sterile surgical knife and placed in a 2-mL screw cap tube with 400 μL sterile distilled H_2O and ~200 mg sterile glass beads (Sigma). Acrylamide pieces were disrupted by vigorous shaking (5000 r.p.m. for 1 min) on a Mini Beadbeater and then the tubes were left at 4 °C overnight. The gel-free DNA (5–10 μL) was reamplified as described previously with non-GC clamped primers (960Fb/1200R). The new PCR product was directly sequenced with the same forward and reverse

primers on an Applied Biosystems ABI Prism 3730 DNA Analyzer (Nevada Genomics Center). Bands that resulted in high quality sequence data (15 in total) were matched via BLAST (Altschul *et al.*, 1997) to the nearest identified sequence in the GenBank database.

16S rRNA gene clone library construction and sequencing

Clone libraries of nearly full-length bacterial 16S rRNA genes (27F and 1492R primers) were constructed for two depths in the ice cover: 4.8 and 15.9 m. PCR products were pooled from five individual reactions, purified, ligated into cloning vectors (TOPO TA cloning kit, Invitrogen), and transformed following the manufacturer's instructions. Clones containing inserts (as indicated by blue/white screening) were isolated (672 clones per depth).

From each library, 192 clones were sequenced unidirectionally. Twenty-three poor quality sequences (determined by ambiguous nucleotide identities) were removed from the analysis. All remaining sequences were checked for chimeras using the BELLEROPHON (Huber *et al.*, 2004) and CHECK_CHIMERA programs (Cole *et al.*, 2003): nine potential chimeras were identified and removed. An additional 37 short sequences (~200 bp) were removed from the 4.8-m library. These sequences had the highest identities to cyanobacteria and were likely an effect of mispriming of the bacterial 27F primer. The short sequences aligned to the 3′ end of cyanobacterial 16S rRNA gene sequences. The remaining dataset contained 315 clone sequences ranging in length from 534 to 716 bp.

The frequency of unique phylotypes was determined by assembling sequences with $>99\%$ similarity using Sequencher (Gene Codes Corp.). Clone library phylotype richness (S_{Chao1}) and coverage (C_{ACE}) estimates were calculated using the web interface created by Kemp & Aller (2004a, b). S_{Chao1} (Chao, 1984; Chao, 1987) is a nonparametric richness estimator and C_{ACE} (Chao *et al.*, 1993; Lee & Chao, 1994) is a coverage estimate defined as the proportion of sequences from phylotypes with <10 clones that occur more than once in a library.

Near-full length, single-stranded 16S rRNA gene sequences were obtained for one representative clone from each unique phylotype. Phylotype sequences were matched via BLAST (Altschul *et al.*, 1997) to the nearest identified sequence in the GenBank nr. and env_nt databases.

Phylogenetic analysis

Vida phylotypes with the highest BLAST identities to *Actinobacteria* and *Gammaproteobacteria* were analyzed for phylogenetic relatedness. Sequences were aligned (using Genetic Data Environment) to the secondary structure of neighboring 16S rRNA gene sequences downloaded from the Ribosomal Database Project. Aligned sequences were analyzed with the maximum likelihood method using fastDNAml (Felsenstein, 1981; Olsen *et al.*, 1994) and a consensus tree was plotted. One hundred bootstraps were run using the fastDNAml_boot script. A mask excluded highly variable regions of the 16S rRNA gene from the analysis.

Nucleotide sequence accession numbers

Sequences generated in this study can be found under accession numbers DQ521452–DQ521569 in GenBank.

Results

The core sections chosen for this study spanned the length of a 15.9-m ice core (collected in 1996) and contained variable amounts of sediments: high amounts of sediments at 4.8 m, some sediment and flaky mat-like material at 6.9 m, fine-grained sand at 11.8 m, clear ice with faint flocculent-like matter at 14.8 m, and clear ice at 15.9 m. Chlorophyll *a* concentrations ranged from 0.3 μg (at 4.8 m) to 1.9 μg (at 11.8 m) per liter of ice core melt water (Table 1). Chlorophyll *a* was very low or nondetectable at 6.9 and 15.9 m (chlorophyll *a* was not measured in the 14.7-m ice section due to a limited amount of melt water). Total cell concentrations recovered from the ice core decreased an order of magnitude from 2.09×10^6 mL^{-1} at 4.8 m to 0.12×10^6 mL^{-1} at 15.9 m (Table 1) and exhibit the highest concentrations in the ice sections with sediments. The average coefficient of variation among the grids counted (5–20) was 44.95, which is high but not uncommon for samples with sedimentary material. The average cell volume across all five depths was 0.06 μm^3 cell^{-1} ($n = 100$, SE 0.01, Table 1), with an average length of 0.62 μm (SE 0.02) and width of 0.35 μm (SE 0.01). Several large diatom frustules were observed at 4.8 and 11.8 m.

Nucleic acid extraction values ranged from 0.37 to 10.16 ng DNA mL^{-1} ice melt water filtered. PCR screening indicated that bacterial and eukaryal DNA was present at all depths studied, whereas no archaeal DNA was amplified from the ice sections studied.

Table 1. Chlorophyll *a* values, total prokaryotic cell counts, and cell volume estimates for the Lake Vida ice cover microbial community

Sample depth (m)	Chlorophyll *a* (μg L^{-1})*	Prokaryote count (cells mL^{-1})*	Avg. biovolume (μm^3 cell^{-1})
4.8	0.3	2.09×10^6	0.043
6.9	0.0	1.33×10^6	0.052
11.8	1.9	0.47×10^6	0.028
14.7	n/a†	0.54×10^6	0.044
15.9	0.0	0.12×10^6	0.083

*Volume of ice melt water.

†Not measured due to insufficient melt water volume.

Eukaryal and bacterial DGGE

Eukaryal DGGE illustrated distinct banding patterns between the depths analyzed (supplementary Fig. S1). One universal phylotype was evident across all of the depths (denoted by the white arrow in supplementary Fig. S1). Phylotype profile pairwise similarities (supplementary Table S1) were low between all depths (similarity values $< 48\%$). The 15.9-m phylotype profile was the least similar (20%) to 4.8 m and most similar (48%) to 14.7 m. The 4.8-m profile was unlike the other depths analyzed (pairwise similarities between 20% and 29%).

Eukaryal DGGE band sequences were predominantly green-algal taxa (Table 2); chlorophyte-related sequences represented one-third of those determined and were present at all depths. Three fungal sequences were identified at 11.8 m. One diatom sequence (98% identity to *Navicula atomus*) was present in the 4.8-m profile. Eight of the band sequences were more than 99% identical to sequences in GenBank. As anticipated, comigrating bands (e.g. PT_ANTLV_31 and PT_ANTLV_11) had identical sequences.

As seen in the eukaryal DGGE, the bacterial DGGE also exhibited differences in phylotype distribution at each of the depths surveyed in the ice cover (supplementary Fig. S2). Four universal phylotypes were evident across all samples (denoted by the white arrows in supplementary Fig. S2). Pairwise similarities (supplementary Table S1) ranged from 32% to 59% across all pairwise depth comparisons. The 15.9-m bacterial phylotype profile was the least similar (36%) to 4.8 m and the most similar (59%) to 14.7 m.

16S rRNA gene sequences

Analysis of the 16S rRNA gene clone libraries included 134 sequences from 4.8 m and 181 from 15.9 m (> 530 bp in length). The 4.8-m library had 66 phylotypes (defined by 99% sequence identity), 52 of which occurred once or twice in the library. The 15.9-m library had 37 phylotypes with 22 representatives occurring once or twice. There were only two cases in which phylotypes from both libraries shared 99% sequence similarity. The consensus sequence of these phylotypes had the highest BLAST identities to *Gillisia limnaea* (affiliated with the *Flavobacteria*, 98% identity, AJ440991) isolated from Antarctic microbial mats and an alpine lake *Betaproteobacterium* (99% identity, AJ867919).

The number of predicted phylotypes (S_{Chao1}) in the 4.8-m ice habitat was 133 (supplementary Fig. S3). The complete sequenced library represented ~50% of the phylotypes predicted to exist in the environment. Coverage estimates (C_{ACE}) indicated that the proportion of sequences from relatively rare phylotypes (with < 10 clones) that occurred more than once in the library was *c.* 63%. The 15.9-m library had substantially lower richness estimates (predicted S_{Chao1} was 48) and higher coverage estimates (C_{ACE} was *c.* 83%). The library represented ~75% of the number of phylotypes predicted to exist in the environment.

Unique phylotypes (near-full length) were grouped by taxonomic distribution based on the closest identified neighbor in GenBank (Table 3). Bacteria grouped within at least 10 different phyla at 4.8 m. *Actinobacteria* dominated the clone library from 4.8 m (42% of clone sequences). The library also contained many *Bacteroidetes* (13%), *Gammaproteobacteria* (10%) and *Cyanobacteria* (8%) sequences. Four phylotypes were most closely related to eukaryal plastid sequences including three *Bacillariophytes* and one *Chlorophyte*. Approximately 36% of the sequences had the highest identities to phylotypes isolated from polar or glacial environments and 5% had the highest identities to phylotypes from marine environments (sea ice, sea water or marine sediments).

Table 2. Affiliation of eukaryal denaturing gradient gel band sequences in the Lake Vida ice cover (see supplementary Fig. S1)

Taxonomic group	18S rRNA gene identification (accession no.)*	% identity	Band name (accession no.)†	Band no.	Sample depth (m)
Apicomplexa	*Colpodella edax* (AY234843)	99	PT_ANTLV_26 (DQ521454)	26	6.9
Bacillariophyta	*Navicula atomus var. permitis* (AJ867024)	98	PT_ANTLV_33 (DQ521452)	33	4.8
Cercozoa	Uncultured rhizosphere cercozoan (AJ506034)	94	PT_ANTLV_27 (DQ521455)	27	6.9
Chlorophyta	*Chlamydomonas incerta* (AY781664)	100	PT_ANTLV_25 (DQ521463)	25	11.8
	Chlorella sp. (AF514413)	100	PT_ANTLV_37 (DQ521457)	37	4.8
	Chlorella sp. Pic 9/21 P-1w (AY197630)	100	PT_ANTLV_31 (DQ521464)	31	6.9
		100	PT_ANTLV_11 (DQ521459)	11	15.9
	Uncultured *Chlorophyta* (AY180047)	100	PT_ANTLV_05 (DQ521458)	5	15.9
Chrysophyceae	Uncultured chrysophyte (DQ104087)	100	PT_ANTLV_15 (DQ521453)	15	14.7
		100	PT_ANTLV_06 (DQ521456)	6	15.9
Chytridiomycota	*Rhizophydium* sp. UGA-F1 (AF164265)	95	PT_ANTLV_22 (DQ521461)	22	11.8
	Uncultured chytridiomycete (AF372719)	98	PT_ANTLV_14 (DQ521460)	14	11.8
Ciliophora	*Frontonia* sp. (AF255359)	97	PT_ANTLV_34 (DQ521465)	34	4.8
	Paramecium calkinsi (AF100310)	96	PT_ANTLV_36 (DQ521466)	36	4.8
Unidentified	Uncultured fungus (AY821997)	97	PT_ANTLV_23 (DQ521462)	23	11.8

*Closest identified neighbor in the NCBI nr database.

†From direct PCR amplification of acrylamide bands.

Table 3. Affiliation of 16S rRNA gene phylotypes at 4.8 and 15.9 m depths in the Lake Vida ice cover

			4.8 m†		15.9 m†	
Taxonomic phylum	Taxonomic class	16S rRNA gene identification (accession no.)*	Phylotype (clones)‡	% identity	Phylotype (clones)‡	% identity
Acidobacteria	*Acidobacteriales*	Bacterium Ellin337 (AF498719)	ANTLV1_C02 (1)	96		
	Unidentified	Uncultured *Acidobacteria* bacterium (AY211077)	ANTLV1_D09 (1)	94		
Actinobacteria	*Actinobacteridae*	Arctic sea ice bacterium ARK10173 (AF468440)			ANTLV7_C06 (1)	99
		Bacterium CS117 (AY124341)	ANTLV1_H11 (3)	99		
		Cellulomonas sp. IFO16240 (AB023361)			ANTLV9_G12 (7)	97
		Cellulomonas sp. TR7-6 (AB166887)	ANTLV2_A07 (3)	98		
		Cryobacterium psychrophilum (AJ544063)			ANTLV7_C10 (1)	98
					ANTLV9_D07 (3)	98
		Curtobacterium sp. VKM Ac-2058 (AB042093)	ANTLV1_D04 (1)	96		
		Frigoribacterium sp. 34/19 (AY571813)	ANTLV2_A11 (2)	97		
		Janibacter-like sp. V4.BO.43 (AJ244674)	ANTLV2_F12 (1)	97		
			ANTLV2_G12 (4)	97		
		Leifsonia rubra (AJ438585)	ANTLV1_B10 (1)	96	ANTLV7_E10 (3)	99
			ANTLV1_H04 (22)	97		
		Luteococcus peritonei (AJ132334)	ANTLV2_C06 (2)	94		
	Unidentified	Antarctic bacterium R-8287 (AJ440992)	ANTLV1_A09 (1)	99	ANTLV9_G02 (1)	98
		Uncultured actinobacterium (AF454303)	ANTLV2_H06 (2)	93	ANTLV9_H09 (1)	95
			ANTLV2_D07 (8)	96		
		Uncultured actinobacterium (AJ575506)	ANTLV2_G03 (3)	96		
		Uncultured actinobacterium (AJ575519)			ANTLV7_H04 (2)	99
		Uncultured actinobacterium (AY743693)	ANTLV2_H11 (1)	96		
		Uncultured bacterium ARFS-6 (AJ277690)	ANTLV1_D11 (2)	95		
		Uncultured eubacterium WCHB1-18 (AF050574)			ANTLV9_C09 (1)	96
Bacteroidetes	*Flavobacteria*	Arctic sea ice bacterium ARK10063 (AF468410)			ANTLV7_G11 (7)	99
		Flavobacterium frigoris (AJ557887)	ANTLV2_D10 (4)	96		
		Flavobacterium hibernum (L39067)	ANTLV1_G11 (1)	96		
		Gillisia limnaea (AJ440991)	ANTLV2_F09 (2)	98	ANTLV7_A10 (3)	98
					ANTLV7_C07 (2)	99
	Sphingobacteria	*Algoriphagus borotolerans* (AB197852)	ANTLV1_B03 (5)	96		
		Arctic sea ice bacterium ARK10280 (AF468433)			ANTLV9_G04 (14)	98
		Cyclobacterium amurskyense (AY960985)			ANTLV9_H08 (2)	97
		Glacier bacterium FJS5 (AY315161)	ANTLV2_B07 (1)	98		
	Unidentified	Antarctic bacterium R-7666 (AJ440984)	ANTLV2_D11 (2)	96		
		Uncultured *Bacteroidetes* bacterium (AJ318142)	ANTLV1_H05 (1)	94		

Table 3. Continued.

			4.8 m†		15.9 m†	
Taxonomic phylum	Taxonomic class	16S rRNA gene identification (accession no.)*	Phylotype (clones)‡	% identity	Phylotype (clones)‡	% identity
		Uncultured *Bacteroidetes* bacterium (AM114443)	ANTLV2_H03 (1)	97		
		Uncultured *Bacteroidetes* bacterium (AY038797)	ANTLV2_E05 (1)	95		
Candidate Division OD1	Unidentified	Uncultured candidate division OD1 bacterium (AY922093)			ANTLV9_G05 (1)	82
Candidate Division TM7	Unidentified	Uncultured candidate division TM7 bacterium (AF445701)			ANTLV9_C10 (6)	93
					ANTLV9_F06 (2)	93
					ANTLV9_G01 (1)	92
					ANTLV9_G11 (9)	93
Chloroflexi	Unidentified	Uncultured *Chloroflexi* bacterium (AY921747)	ANTLV1_C12 (1)	88		
Cyanobacteria	*Oscillatoriales*	*Limnothrix redekei* CCAP 1443/1 (AJ580007)	ANTLV1_B04 (1)	95		
			ANTLV1_H09 (1)	98		
			ANTLV2_C09 (4)	98		
		Microcoleus vaginatus (AF284803)	ANTLV1_A12 (1)	97		
		Tychonema bourrellyi (AB045897)	ANTLV1_A05 (1)	98		
	Unidentified	Uncultured Antarctic cyanobacterium (AY151726)	ANTLV2_A01 (2)	97		
		Uncultured Antarctic cyanobacterium (AY151728)	ANTLV1_H06 (1)	99		
Firmicutes	*Bacillales*	*Virgibacillus necropolis* (AJ315056)			ANTLV9_F07 (3)	98
	Clostridia	*Clostridium bowmanii* (AJ506119)	ANTLV1_E02 (1)	99		
		Uncultured *Clostridiaceae* bacterium (AY395437)	ANTLV1_C11 (1)	99		
Gemmatimonadetes	Unidentified	Uncultured *Gemmatimonadetes* bacterium (AY124345)	ANTLV1_E09 (1)	98		
Planctomycetes	*Planctomycetacia*	Gemmata-like str. CJuql4 (AF239693)	ANTLV2_E09 (1)	94		
		Uncultured *planctomycete* (AJ616284)	ANTLV2_C10 (1)	98		
Proteobacteria	*Alphaproteobacteria*	*Agrobacterium sanguineum* (AB062105)	ANTLV1_B09 (1)	99		
		Brevundimonas sp. FWC04 (AJ227793)	ANTLV1_C06 (1)	99		
			ANTLV1_E07 (2)	99		
		Catellibacterium nectariphilum (AB101543)	ANTLV1_G08 (1)	96		
		Rhodobacter sp. 1-5 (AF513400)			ANTLV7_G07 (2)	99
					ANTLV9_B12 (3)	95
		Sphingomonas sp. SIA181-1A1 (AF395032)	ANTLV1_E12 (1)	97		
			ANTLV1_H10 (2)	97		
		Uncultured *Alphaproteobacterium* (AY947933)	ANTLV2_E06 (2)	95		
	Betaproteobacteria	Antarctic bacterium R-7724 (AJ440986)			ANTLV7_G03 (3)	98
		Betaproteobacterium Wuba72 (AF336361)	ANTLV1_D07 (1)	98	ANTLV9_A06 (6)	98
		Leptothrix sp. S1.1 (DQ241397)	ANTLV2_E04 (1)	97		
		Uncultured *Betaproteobacterium* (AJ867919)	ANTLV1_B06 (1)	99	ANTLV7_H07 (1)	99

Table 3. Continued.

Taxonomic phylum	Taxonomic class	16S rRNA gene identification (accession no.)*	4.8 m† Phylotype (clones)‡	4.8 m† % identity	15.9 m† Phylotype (clones)‡	15.9 m† % identity
		Uncultured *Betaproteobacterium* (AY509483)	ANTLV2_C11 (1)	97		
		Uncultured *Betaproteobacterium* (AY947997)	ANTLV1_G06 (1)	98		
	Epsilonproteobacteria	Uncultured *Epsilonproteobacterium* (AB015558)	ANTLV2_G05 (1)	89		
		Uncultured *Epsilonproteobacterium* 1063 (AB030613)	ANTLV2_G07 (1)	97		
		Unidentified *Epsilonproteobacterium* (AB015535)			ANTLV7_F08 (1)	97
	Gammaproteobacteria	Antarctic bacterium R-9035 (AJ441002)			ANTLV9_C12 (4)	99
		Aquamonas fontana (AB120965)	ANTLV1_A07 (2)	97		
		Arctic sea ice bacterium ARK10244 (AF468401)			ANTLV9_A11 (57)	99
		Gammaproteobacterium MN 154.3 (AJ313020)			ANTLV7_H08 (1)	88
		Gas vacuolate str. S36-W < gv > 1 (U14584)			ANTLV7_A07 (23)	98
					ANTLV9_B08 (2)	98
		Marinobacter maritimus (AJ704395)			ANTLV7_D09 (2)	97
		Pseudomonas sp. ARCTIC-P23 (AY573032)			ANTLV7_H01 (1)	98
		Pseudomonas sp. ARCTIC-P33 (AY573033)			ANTLV9_D02 (1)	96
		Stenotrophomonas maltophilia (AJ131114)	ANTLV1_F11 (1)	99		
		Uncultured *Gammaproteobacterium* (AF445683)	ANTLV2_E01 (3)	98		
			ANTLV2_E03 (1)	97		
			ANTLV2_F07 (3)	94		
		Uncultured *Gammaproteobacterium* (AJ704666)			ANTLV7_H05 (1)	93
		Uncultured *Gammaproteobacterium*(AY922114)	ANTLV1_A02 (3)	96		
		Uncultured *Gammaproteobacterium* (DQ234120)			ANTLV9_C04 (1)	97
		Uncultured *Gammaproteobacterium* Sva0318 (AJ240989)			ANTLV9_H04 (2)	95
		Uncultured *Xanthomonas* sp. (AY571839)	ANTLV1_C08 (1)	99		
	Unidentified	Uncultured proteobacterium (AF491673)	ANTLV2_G10 (1)	90		
Verrucomicrobia	Unidentified	Uncultured *Verrucomicrobia* bacterium (AF465651)	ANTLV1_D01 (1)	94		
Eukaryotic plastids	*Bacillariophyta*	*Thalassiosira pseudonana* mitochondrion (DQ186202)	ANTLV2_A04 (1)	87		
		Bacillaria paxillifer chloroplast 16S rRNA gene (AJ536452)	ANTLV1_E03 (3)	97		
			ANTLV2_F08 (3)	97		
	Chlorophyta	*Chlorella saccharophila* chloroplast 16S rRNA gene (D11348)	ANTLV1_D06 (1)	98		

*Closest identified neighbor in either the NCBI nr or env_nt database.

†Phylotype sequence lengths range from 1359 to 1556 bp and clone sequence lengths range from 534 to 716 bp.

‡Phylotype name (number of clone sequences in phylotype).

Sequences in the 15.9-m library grouped within six different bacterial phyla. The library was dominated by *Gammaproteobacteria* (52% of clone sequences). The library also contained many *Bacteroidetes* (15%), *Actinobacteria* (11%), and sequences from the candidate division TM7 (10%). Approximately 72% of the sequences from the 15.9-m library had the highest identities to phylotypes isolated from polar or glacial environments, including Arctic and Antarctic sea ice (Gosink & Staley, 1995; Brinkmeyer *et al.*, 2003), Antarctic microbial mats (Van Trappen *et al.*, 2002), and an Antarctic pond (Reddy *et al.*, 2003). Roughly 65% of the sequences had the highest identities to phylotypes from marine environments.

Gammaproteobacteria and *Actinobacteria* phylogeny

Phylogenetic trees were constructed from Lake Vida ice-associated *Gammaproteobacteria* and *Actinobacteria* 16S rRNA gene sequences based on maximum likelihood analysis. Many 15.9-m ice *Gammaproteobacteria* phylogenetically clustered with sequences from Antarctic and Arctic environments (Fig. 2), including an Antarctic *Marinobacter* isolate from Lake Bonney that is psychrophilic, moderately halophilic, and capable of denitrification (*Marinobacter* sp. ELB17, Ward & Priscu, 1997). Sequences from the 4.8-m library were phylogenetically distinct from the 15.9-m sequences and were more closely related to *Xanthomonas* and *Stenotrophomonas* sequences.

The Lake Vida ice-associated actinobacterial 16S rRNA gene sequences grouped with a broad range of distantly related clusters (Fig. 3). Phylotypes from the 4.8- and 15.9-m libraries clustered closely with each other in several cases. Many of the *Actinobacteria* from Lake Vida ice shared close relationships with other Arctic and Antarctic organisms, including organisms associated with Antarctic microbial mats (from Lake Fryxell and Grace Lake) and polar sea ice. Several phylotypes were phylogenetically related to the Antarctic psychrophile *Leifsonia rubra* (AJ438585, Reddy *et al.*, 2003), including the three phylotypes (representing 26 clones) with *L. rubra* as their nearest identified neighbor in GenBank. This group also contained the marine actinobacterium PHSC20C (Murray *et al.*, 2006) and represents a cold-adapted lineage found in both marine and freshwater environments.

Discussion

Distribution of Lake Vida biota

Bacterial numbers in the Lake Vida ice were comparable to data obtained from other studies of microorganisms in ice. Total bacterial cell counts in the Vida ice core (0.12×10^6–2×10^6 cells mL^{-1}) are comparable to those found in many polar environments, including cryoconite holes (C.H. Fritsen, pers. commun.), Arctic sea ice (e.g. Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003), Antarctic sea ice (e.g. Helmke & Weyland, 1995; Gowing *et al.*, 2004), and the ice covers and water columns (Ward & Priscu, 1997; Priscu *et al.*, 1998, respectively) of perennially ice-covered lakes. The Lake Vida total bacterial cell counts were orders of magnitude higher than bacterial numbers (10^2–10^3) in Lake Vostok accretion ice (Karl *et al.*, 1999; Priscu *et al.*, 1999), which is covered by several thousand meters of glacial ice. Lake Vida ice and Greenland ice (Sheridan *et al.*, 2003; Miteva & Brenchley, 2005) cells were similar in size ($< 0.1\ \mu m^3$). These small cell sizes may be an adaptation to stresses induced within the ice (Fogg, 1998), including nutrient limitation via reductive cell division or adaptation to oligotrophy.

Putative activity

The 16S rRNA gene similarity of many Vida sequences (178) and isolates (e.g. seven that are related to *Cryobacterium psychrophilum*; data not shown) to organisms isolated from polar or glacial environments is not direct evidence for *in situ* activity. However, a previous study reported detectable primary production and thymidine incorporation in ice melt water throughout a 15-m Lake Vida ice core with the highest rates in the upper 4 m (Doran *et al.*, 2003). The abundance of chlorophyll *a* at 4.8 m and presence of cyanobacteria at 4.8 and 6.9 m (as evidenced by representation in the 4.8-m clone library and culturing; data not shown) supports the potential for photoautotrophic primary production above 6.9 m in the ice cover. In ice covers, photosynthetically available radiation is attenuated by high gas bubble density and entrained sediment (McKay *et al.*, 1994; Fritsen & Priscu, 1999). This does not necessarily mean that the phototrophic cells are inactive. Light saturation of photosynthesis occurs at low irradiances (30 μmol photons m^{-2} s^{-3}) within Lake Bonney (Neale & Priscu, 1998) and phytoplankton exhibit mixotrophy in Lake Fryxell and Lake Hoare (Roberts & Laybourn-Parry, 1999; Marshall & Laybourn-Parry, 2002). Thus, phototrophs in Lake Vida ice may photosynthesize below 6 m in low light conditions or may exhibit heterotrophy.

Bacteroidetes was one of the most abundant groups in both of the Lake Vida ice clone libraries and may be major contributors of heterotrophic activity in the ice cover. *Bacteroidetes* play an important role in remineralization processes in aquatic systems (Kirchman, 2002) and may also be important in ice systems. Some *Bacteroidetes* can utilize cellulose, chitin, DNA, lipids, or proteins that would be trapped within the ice after cell decay. Many of the Vida sequences had nearest identified neighbors to *Bacteroidetes* from polar environments, including the psychrotroph

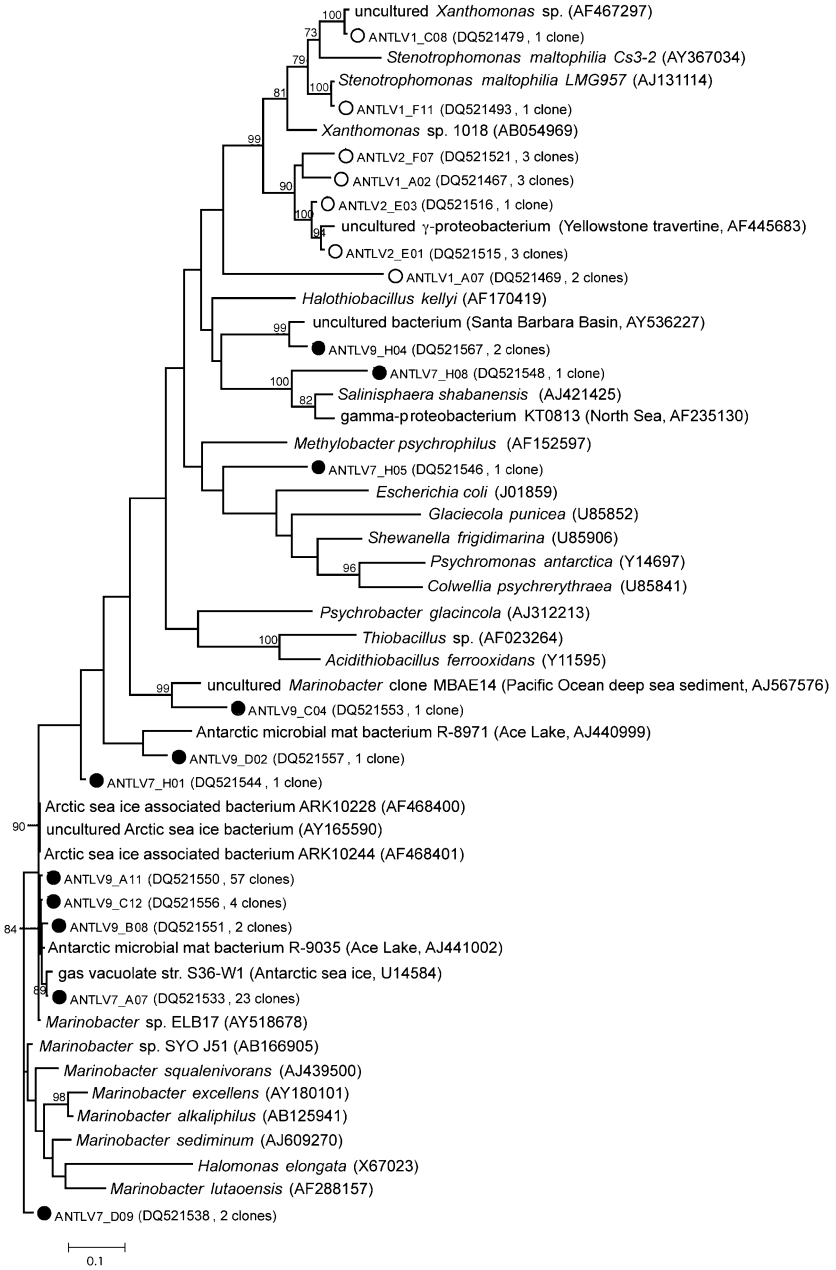

Fig. 2. Maximum likelihood phylogenetic tree showing the affiliation of Lake Vida *Gamma-proteobacteria* 16S rRNA gene sequences. Phylotypes from Lake Vida are indicated with open circles for the 4.8-m library and closed circles for the 15.9-m library. Bootstrap values greater than 50 are shown.

Flavobacterium hibernum isolated from a freshwater lake of the Vestfold Hills region of Antarctica (Crooked Lake; McCammon *et al.*, 1998). *Flavobacterium hibernum* may have a bacteriovorous role in the environment because of its ability to glide on surfaces and to lyse gram-negative cells (McCammon *et al.*, 1998).

Diversity of biota found within ice

The variety of eukaryal phylotypes in the bottom portions of the ice cover was somewhat unanticipated. Microscopic enumeration of eukaryotes was not performed due to the low availability of ice core melt water, though a few eukaryal cells were observed during prokaryotic cell counts. It is uncertain whether the amplified eukaryal DNA originated from intact cells or was free within the ice. Samples for nucleic acid extraction were collected on 0.2-μm filters, suggesting that the cellular, and not dissolved, fraction was analyzed. If the recovered eukaryal DNA originated from intact cells, these organisms may have survival mechanisms for withstanding cryogeny. Some fungi, for instance, are stress tolerant and have lower minimal water activities for growth than prokaryotes (Onofri *et al.*, 2004). Many Antarctic fungi have shown resistance to low temperatures, high

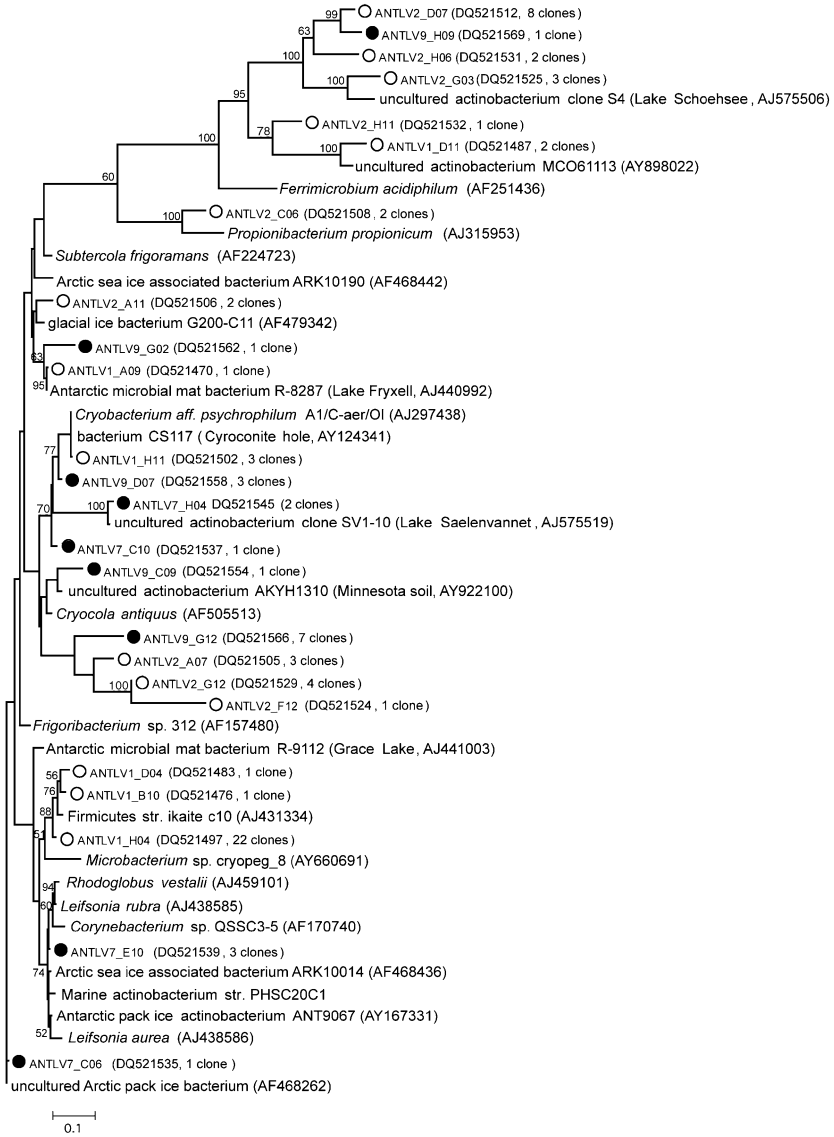

Fig. 3. Maximum likelihood phylogenetic tree showing the affiliation of Lake Vida *Actinobacteria* 16S rRNA gene sequences. Phylotypes from Lake Vida are indicated with open circles for the 4.8-m library and closed circles for the 15.9-m library. Bootstrap values greater than 50 are shown.

UV, high ionizing radiation, and desiccation (Onofri *et al.*, 2004). Alternatively, eukaryal DNA may have been recovered from intact cells that were not alive but instead lifeless and frozen within the ice matrix. Endogenous nucleases that normally degrade DNA after a cell dies can be destroyed or inactivated by low temperatures, high salinity, and rapid desiccation (Hofreiter *et al.*, 2001), thereby slowing the rate of DNA decay within ice.

Although bacteria and eukarya are prevalent, archaea are not commonly detected in ice environments. Archaeal 16S rRNA gene primers did not amplify rRNA gene from Vostok accretion ice (Lyons *et al.*, 1998; Christner *et al.*, 2001), ancient glacial ice (Christner *et al.*, 2003b), Arctic and Antarctic sea ice (Brown & Bowman, 2001), and Vida ice. However, archaea have been documented in young, seasonal Antarctic and Arctic sea ice (Stewart *et al.*, 2003; Junge *et al.*, 2004). There are several suppositions explaining the apparent absence of archaea in older ice, including the possibility that archaea are out-competed by other prokaryotes in the ice or that the archaea are more prone to death and decay once entombed within the ice.

Our results indicate that the 4.8-m bacterial assemblage was more diverse than the 15.9-m assemblage with no 100% similar phylotypes found at both depths. We found that the sensitivity of detection with DGGE is somewhat limited in rich assemblages with many rare phylotypes (e.g. the 4.8-m ice) and is more robust in a less rich and more evenly distributed assemblage such as that seen in the 15.9-m ice. To the best of our knowledge, richness has not been calculated from other ice environment's including cryoconites,

glacial ice, permanent lake ice, and sea ice. Richness estimates calculated for the water column of Lake Bonney based on unique restriction fragment length polymorphism (RFLP) patterns (20–392 phylotypes; Glatz *et al.*, 2006) were within the range seen in the Vida ice libraries. Richness estimates were also calculated for low temperature marine sediments: 541-1128 phylotypes in Antarctic sediments from the continental shelf (Bowman & McCuaig, 2003) and 2716 phylotypes in Arctic marine sediments (Kemp & Aller, 2004a, b). As expected, richness for the Vida libraries is low in comparison with sediment environments.

PCR biases (Reysenbach *et al.*, 1992; Suzuki & Giovannoni, 1996; Suzuki *et al.*, 1998) and multiple rRNA gene copies per cell (Klappenbach *et al.*, 2000) can affect the distribution and relative frequency of phylotypes within clone libraries. Therefore, the abundance of *Gammaproteobacteria* (*c.* 50% of the sequences) in the 15.9-m Vida clone library elicits guarded interpretation (as does the relative abundance data for both libraries). It is also important to note that the Vida 15.9-m library was estimated to have a high percent coverage of the natural community – roughly 70% of the community was described with only 50 clone sequences. Though the Vida 16S rRNA gene clone libraries are potentially subject to biases, *Gammaproteobacteria* were also abundant in several other polar studies, including Arctic (Junge *et al.*, 2002; Brinkmeyer *et al.*, 2003) and Antarctic sea ice (Bowman *et al.*, 1997; Brinkmeyer *et al.*, 2003) and the water column of Lake Bonney (Glatz *et al.*, 2006). These data suggest that certain *Gammaproteobacteria* (e.g. *Colwellia* and *Marinobacter*) are common among these habitats.

Bacteria isolated from ice cores have likely endured a number of environmental stresses (including freezing, desiccation, and starvation). Therefore, spore-forming bacteria are expected to be present in large numbers in ice environments. Interestingly, the Vida ice clone libraries had only a few representatives of known endospore-forming genera (two phylotypes at 4.8 m and one at 15.9 m). However, both of the libraries contained many *Actinobacteria*-related (high G+C, gram positives) sequences (~25% of unique phylotypes detected in each library). Similar trends were also seen in a Greenland glacier (Sheridan *et al.*, 2003; Miteva *et al.*, 2004) and an Antarctic cryoconite hole (Christner *et al.*, 2003a). Willerslev *et al.* (2004) showed that *Actinobacteria* outlast low G+C gram-positive and gram-negative cells in old permafrost, shifting from *c.* 30% to 100% of the taxonomic distribution of clones after 300 000–600 000 years. They concluded that this group has survival mechanisms that are not fully understood at this point but which may include transition to resting forms with low level metabolic activity (i.e. anabiosis; Mulyukin *et al.*, 2001). The 15.9-m library also had many representatives of candidate division TM7, which contain gram-positive cell envelopes (Hugenholtz *et al.*, 2001).

Source and origin

While a few taxa may be ubiquitous in the Lake Vida ice cover (e.g. *Chlorella* sp. as evident by the universal eukaryal DGGE band), the low DGGE pairwise similarities between the depths and the depth-specific phylotypes are indicative of differences in ice habitability (i.e. the ability of the physical-chemical environment within the ice to sustain life) or organismal origin in the upper and lower portions of ice cover. The source of the organisms in the upper ice is thought to be a combination of modern aeolian deposition and glacial melt water that flows through streams and sediments before flooding the ice cover. Thus, it is not surprising that the biotic community in the 4.8-m ice contains many bacteria and eukarya that have high similarities to organisms from soil and freshwater habitats.

The bottom of the Vida ice cover is likely to have fluctuated through periods of melting and freezing over the 2800-year period of its presumed existence. Cycling of the thickness and mass of the ice cover is likely controlled by winter and summer fluctuations that determine the net annual mean temperature at the bottom of the ice cover. Despite the present-day seasonal fluctuations, thermodynamic modeling suggests a net annual accretion of ice (Doran *et al.*, 2003). Net accretions at the bottom further imply that ice near the bottom is expected to be of lake water origin. The significant increase in chloride concentrations in the 15.9-m ice (Doran *et al.*, 2003) supports the notion that this ice formed at or in the transition from a freshwater water column to one with higher dissolved salts (Doran *et al.*, 2003). If the 15.9-m ice did indeed form from the water column of the lake, the biota found in the deep ice may also be present in the lake itself, including a diverse array of bacteria and eukaryotes. The high percentage (~65%) of clone sequences in the 15.9-m library with the highest identities to sequences from marine environments (compared to only ~5% at 4.8 m) suggests that these organisms may be able to sustain activity or viability within the high salinity of the water column.

Christner *et al.* (2001) suggest that organisms found in the Lake Vostok (Antarctica) accretion ice regularly seed the water column of the lake. Similarly, the organisms found at the bottom of the Vida ice cover may seed the water column during periods of ice melt. The population of microorganisms existing within the deep ice has the potential to seed the underlying brine pocket of Lake Vida during periods of bottom-ice ablation. Thus, a dynamic exchange of biota may occur between the water column and deep ice during melting and freezing cycles at the bottom of the ice cover, where the lake water and ice are both seeds and sinks.

Conclusion

A diverse range of bacteria and eukarya are present at all depths in the Lake Vida ice cover. The conundrum of

whether these organisms are active within the ice has not been solved. The data suggest that there are differences in ice habitability or organismal origin in the upper and lower portions of the ice cover. We suggest that the 4.8-m ice community has a terrestrial origin (e.g. McMurdo Dry Valleys soil and freshwater) and that the 15.9-m community has likely been derived from the water column. A dynamic exchange of biota may occur between the water column and deep ice when the bottom of the ice cover melts or freezes. The Lake Vida water column may be a refuge from the harsh environmental conditions within the McMurdo Dry Valleys.

Acknowledgements

This work was supported by: Nevada Space Grant Consortium, Desert Research Institute Research Foundation, NASA ASTEP (Grant NAG5-12889), NASA EPSCoR (Grant NCC5-583), NSF LExEN (Grant OPP-9814972), and NSF Office of Polar Programs. We would like to thank B. Carter and J. Grzymski for their assistance during stages of this research as well as P. Doran and J. Priscu for providing the initial efforts in obtaining the Lake Vida ice core.

References

Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W & Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Bowman JP & McCuaig RD (2003) Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. *Appl Environ Microbiol* **69**: 2463–2483.

Bowman JP, McCammon SA, Brown MV, Nichols DS & McMeekin TA (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**: 3068–3078.

Brinkmeyer R, Knittel K, Jurgens J, Weyland H, Amann R & Helmke E (2003) Diversity and structure of bacterial communities in arctic versus antarctic pack ice. *Appl Environ Microbiol* **69**: 6610–6619.

Brown MV & Bowman JP (2001) A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). *FEMS Microbiol Ecol* **35**: 267–275.

Calkin PE & Bull C (1967) Lake Vida, Victoria Valley Antarctica. *J Glaciol* **6**: 833–836.

Chao A (1984) Nonparametric-estimation of the number of classes in a population. *Scand J Stat* **11**: 265–270.

Chao A (1987) Estimating the population-size for capture recapture data with unequal catchability. *Biometrics* **43**: 783–791.

Chao A, Ma MC & Yang MCK (1993) Stopping rules and estimation for recapture debugging with unequal failure rates. *Biometrika* **80**: 193–201.

Christner BC, Mosley-Thompson E, Thompson LG & Reeve JN (2001) Isolation of bacteria and 16S rDNA's from Lake Vostok accretion ice. *Environ Microbiol* **3**: 570–577.

Christner BC, Kvitko BH & Reeve JN (2003a) Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles* **7**: 117–183.

Christner BC, Mosley-Thompson E, Thompson LG & Reeve JN (2003b) Bacterial recovery from ancient glacial ice. *Environ Microbiol* **5**: 433–436.

Cole JR, Chai B, Marsh TL *et al.* (2003) The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. *Nucleic Acids Res* **31**: 442–443.

DeLong EF (1992) Archaea in coastal marine environments. *Proc Natl Acad Sci* **89**: 5685–5689.

Diez B, Pedros-Alio C, Marsh T & Massana R (2001) Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. *Appl Environ Microbiol* **67**: 2942–2951.

Doran PT, Fritsen CF, McKay CP, Priscu JC & Adams EE (2003) Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "ice-sealed" east Antarctic lake. *Proc Natl Acad Sci* **100**: 26–31.

Erlich HA, Gelfand D & Sninsky JJ (1991) Recent advances in the polymerase chain-reaction. *Science* **252**: 1643–1651.

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**: 368–376.

Fogg GE (1998) *The Biology of Polar Habitats*. Oxford University Press, New York.

Fritsen CH & Priscu JC (1998) Cyanobacterial assemblages in permanent ice covers on Antarctic lakes: distribution, growth rate, and temperature response of photosynthesis. *J Phycol* **34**: 587–597.

Fritsen CH & Priscu JC (1999) Seasonal change in the optical properties of the permanent ice cover on Lake Bonney, Antarctica: consequences for lake productivity and phytoplankton dynamics. *Limnol Oceanogr* **44**: 447–454.

Gast RJ, Dennett MR & Caron DA (2004) Characterization of protistan assemblages in the Ross Sea, Antarctica, by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **70**: 2028–2037.

Glatz RE, Lepp PW, Ward BB & Francis CA (2006) Planktonic microbial community composition across steep physical/chemical gradients in permanently ice-coverd Lake Bonney, Antarctica. *Geobiology* **4**: 53–67.

Gosink JJ & Staley JT (1995) Biodiversity of gas vacuolate bacteria from Antarctic sea-ice and water. *Appl Environ Microbiol* **61**: 3486–3489.

Gowing MM, Garrison DL, Gibson AH, Krupp JM, Jeffries MO & Fritsen CH (2004) Bacterial and viral abundance in Ross Sea summer pack ice communities. *Mar Ecol Prog Ser* **279**: 3–12.

Helmke E & Weyland H (1995) Bacteria in sea-ice and underlying water of the eastern Weddell Sea in midwinter. *Mar Ecol Prog Ser* **117**: 269–287.

Hobbie JE, Daley RJ & Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* **33**: 1225–1228.

Hofreiter M, Serre D, Poinar HN, Kuch M & Paabo S (2001) Ancient DNA. *Nat Rev Genet* **2**: 353–359.

Huber T, Faulkner G & Hugenholtz P (2004) Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20**: 2317–2319.

Hugenholtz P, Tyson GW, Webb RI, Wagner AM & Blackall LL (2001) Investigation of candidate division TM7, a recently recognized major lineage of the domain bacteria with no known pure-culture representatives. *Appl Environ Microbiol* **67**: 411–419.

Junge K, Imhoff F, Staley T & Deming JW (2002) Phylogenetic diversity of numerically important Arctic sea-ice bacteria at subzero temperature. *Microb Ecol* **43**: 315–328.

Junge K, Eicken H & Deming JW (2004) Bacterial activity at −2 to −20 degrees C in Arctic wintertime sea ice. *Appl Environ Microbiol* **70**: 550–557.

Kane MD, Poulsen LK & Stahl DA (1993) Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide hybridization probes designed from environmentally derived 16S rRNA sequences. *Appl Environ Microbiol* **59**: 682–686.

Karl DM, Bird DF, Bjorkman K, Houlihan T, Shackelford R & Tupas L (1999) Microorganisms in the accreted ice of Lake Vostok, Antarctica. *Science* **286**: 2144–2147.

Kemp PF & Aller JY (2004a) Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. *FEMS Microbiol Ecol* **47**: 161–177.

Kemp PF & Aller JY (2004b) Estimating prokaryotic diversity: When are 16S rDNA libraries large enough? *Limnol Oceanogr: Methods* **2**: 114–125.

Kirchman DL (2002) The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol Ecol* **39**: 91.

Klappenbach JA, Dunbar JM & Schmidt TM (2000) rRNA operon copy number reflects ecological strategies of bacteria. *Appl Environ Microbiol* **66**: 1328–1333.

Lane DJ (1991) *16S/23S rRNA Sequencing* (Stackebrandt E & Goodfellow M, eds), pp. 130–141. Wiley Press, Chichester.

Lee SM & Chao A (1994) Estimating population-size via sample coverage for closed capture-recapture models. *Biometrics* **50**: 88–97.

Lin E, Caron D & DeLong E (1996) Development and field application of a quantitative method for examining natural assemblages of protists with oligonucleotide probes. *Appl Environ Microbiol* **62**: 1416–1423.

Lyons WB, Tyler SW, Wharton RA, McKnight DM & Vaughn BH (1998) A late Holocene desiccation of Lake Hoare and Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Antarct Sci* **10**: 247–256.

Marshall W & Laybourn-Parry J (2002) The balance between photosynthesis and grazing in Antarctic mixotrophic cryptophytes during summer. *Freshwater Biol* **47**: 2060–2070.

Massana R, Murray AE, Preston CM & DeLong EF (1997) Vertical distribution and phylogenetic characterization of marine planktonic Archaea in the Santa Barbara Channel. *Appl Environ Microbiol* **63**: 50–56.

Matsumoto GI (1993) *Geochemical Features of the McMurdo Dry Valley Lakes, Antarctica*, *Vol. 59* (Green WJ & Friedmann EI, eds), pp. 95–118. American Geophysical Union, Washington, DC.

McCammon SA, Innes BH, Bowman JP, Franzmann PD, Dobson SJ, Holloway PE, Skerratt JH, Nichols PD & Rankin LM (1998) *Flavobacterium hibernum* sp. nov., a lactose-utilizing bacterium from a freshwater Antarctic lake. *Int J Syst Bacteriol* **48**: 1405.

McKay CP, Clow GD, Andersen DT & Wharton RAJ (1994) Light transmission and reflection in perennially ice-covered Lake Hoare, Antarctica. *J Geophys Res* **99**: 20427–20444.

Miteva VI & Brenchley JE (2005) Detection and isolation of ultrasmall microorganisms from a 120,000-year-old Greenland glacier ice core. *Appl Environ Microbiol* **71**: 7806–7818.

Miteva VI, Sheridan PP & Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. *Appl Environ Microbiol* **70**: 202–213.

Mulyukin AL, Demkina EV, Kozlova AN, Soina VS & El'-Registan GI (2001) Synthesis of anabiosis autoinducers by non-sporeforming bacteria as a mechanism regulating their activity in soil and subsoil sedimentary rocks. *Microbiology* **70**: 535–541.

Murray AE, Hollibaugh JT & Orrego C (1996) Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Appl Environ Microbiol* 2676–2680.

Murray A, Ferriera S, Johnson J *et al.* (2006) Marine actinobacterium PHSC20C1 genome, direct GenBank submission.

Muyzer G, de Waal EC & Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59**: 695–700.

Neale PJ & Priscu JC (1998) *Fluorescence Quenching in Phytoplankton of the McMurdo Dry Valley Lakes (Antarctica): Implications for the Structure and Function of the Photosynthetic Apparatus*, *Vol. 72* (Priscu J, ed), pp. 241–253. American Geophysical Union, Washington, DC.

Olsen GJ, Matsuda H, Hagstrom R & Overbeek R (1994) fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**: 41–48.

Onofri S, Selbmann L, Zucconi L & Pagano S (2004) Antarctic microfungi as models for exobiology. *Planet Space Sci* **52**: 229–237.

Pospiech A & Neumann B (1995) A Versatile Quick-Prep of Genomic DNA from Gram-Positive Bacteria. *Trends Genet* **11**: 217–218.

Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, McKay CP, Doran PT, Gordon DA, Lanoil BD & Pinckney JL (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. *Science* **280**: 2095–2098.

Priscu JC, Adams EE, Lyons WB *et al.* (1999) Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. *Science* **286**: 2141–2144.

Reddy GSN, Prakash JSS, Srinivas R, Matsumoto GI & Shivaji S (2003) *Leifsonia rubra* sp. nov. and *Leifsonia aurea* sp. nov., psychrophiles from a pond in Antarctica. *Int J Syst Evol Microbiol* **53**: 977–984.

Reysenbach AL, Giver LJ, Wickham GS & Pace NR (1992) Differential Amplification of Ribosomal-RNA Genes by Polymerase Chain-Reaction. *Appl Environ Microbiol* **58**: 3417–3418.

Roberts EC & Laybourn-Parry J (1999) Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. *Freshwater Biol* **41**: 737–746.

Sheridan PP, Miteva VI & Brenchley JE (2003) Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core. *Appl Environ Microbiol* **69**: 2153–2160.

Simon M & Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. *Mar Ecol Prog Ser* **51**: 201–213.

Stackebrandt E & Brambilla E (2002) *Life in Cold Lakes* (Horneck G & Baumstark-Khan C, eds), pp. 161–168. Springer, Berlin.

Stewart FJ, Fritsen CH, Murray AE & Carter BJ (2003) *Prokaryotic Diversity in Antarctic Sea Ice: Inter-Habitat Variability. American Society of Limnology and Oceanography 2003 Aquatic Sciences Meeting: The Earth's Eyes: Aquatic Sciences Through Space and Time*. Salt Lake City, Utah.

Suzuki MT & Giovannoni SJ (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* **62**: 625–630.

Suzuki M, Rappe MS & Giovannoni SJ (1998) Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Appl Environ Microbiol* **64**: 4522–4529.

Takamatsu N, Kato N, Matsumoto GI & Torii T (1998) The origins of salts in water bodies of the McMurdo Dry Valleys. *Antarct Sci* **10**: 439–448.

Van Trappen S, Mergaert J, Van Eygen S, Dawyndt P, Cnockaert MC & Swings J (2002) Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. *Syst Appl Microbiol* **25**: 603–610.

Voytek MA, Priscu JC & Ward BB (1999) The distribution and relative abundance of ammonia-oxidizing bacteria in lakes of the McMurdo Dry Valley, Antarctica. *Hydrobiologia* **401**: 113–130.

Ward BB & Priscu JC (1997) Detection and characterization of denitrifying bacteria from a permanently ice-covered Antarctic lake. *Hydrobiologia* **347**: 57–68.

Welschmeyer N (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol Oceanogr* **38**: 1985–1992.

Wharton RAJ, McKay CP, Clow GD & Andersen DT (1993) *Perennial Ice Covers and their Influence on Antarctic Lake Ecosystems*, *Vol. 59* (Green WJ & Friedmann EI, eds), pp. 53–70. American Geophysical Union, Washington, DC.

Willerslev E, Hansen AJ, Ronn R, Brand TB, Barnes I, Wiuf C, Gilichinsky D, Mitchell D & Cooper A (2004) Long-term persistence of bacterial DNA. *Curr Biol* **14**: R9–R10.

Supplementary material

The following supplementary material is available for this article online.

Table S1. Eukaryal and bacterial DGGE profile pairwise similarities (Sorenson's index).

Fig. S1 Eukaryal 18S rRNA gene denaturing gradient electrophoresis gel showing distribution of phylotypes at different depths in the ice cover. Total number of bands per sample (as determined by GelComparII and visually confirmed) listed at the bottom of each lane. Numbers to the left of individual bands indicate bands that were excised and sequenced. Arrow pointing to the only universal phylotype (note low intensity in some samples).

Fig. S2 Bacterial 16S rRNA gene denaturing gradient electrophoresis gel showing distribution of phylotypes at different depths in the ice cover. Total number of bands per sample (as determined by GelComparII and visually confirmed) listed at the bottom of each lane. Arrows pointing to universal phylotypes (note low intensity in some samples).

Fig. S3 16S rRNA gene clone library richness and coverage estimates. Predicted S_{Chaol} values (panel A) and predicted C_{ACE} values (panel B) for the Lake Vida 4.8 m (filled circles) and 15.9 m (open circles) clone libraries.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6968.2006.00220.x (This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.